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Utilization of Analytical Critical Fluid Instrumentation in Non-Analytical Applications

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Instrumentation systems incorporating critical fluid technology have been developed to a relatively sophisticated level, ranging from micro-extractors of several milliliters to larger capacity units approaching multi-liter capacity. Unlike supercritical fluid chromatography (SFC), the non-analytical uses of such instrumentation are not always recognized, appearing in an array journals and applications. Analytical supercritical fluid extraction (SFE) systems have not always been designed for such use, but can be modified with a modest amount of effort for this purpose. In this presentation, non-analytical applications of the instrumentation for conducting reactions, performing fractionations, evaluating the efficacy of adsorbents and catalysts, and the screening of cosolvents will be documented with examples from our own research studies. A wide variety of reaction chemistries can be evaluated in critical fluid media using analytical extraction equipment. Successful examples from our studies include various types of lipid modification reactions, such as esterification, transesterification, glycerolysis, and acylation. Both manual and automated units have been employed for the above purposes, including their use in evaluating immobilized lipases and ion exchange media as catalysts. Flow reactors and batch systems will be described for the synthesis of vitamin E, sterol esters, and specific enantiomers as products. Extensions of this approach for running reactions in near critical fluids or pressurized liquids are possible, using conventional or modified instrumentation. The use of multiple pumping systems and reactors also allows multi-step synthesis of compounds to be demonstrated in supercritical media. Likewise, extraction and fractionation conditions can be rapidly optimized with the aid of analytical extraction instrumentation. Using automated sequential analyzers, it is possible to screen for the effect of pressure, temperature, and cosolvent in a combinatorial fashion which substantially reduces the time and effort associated with such experiments. Assessment of sorbent selectivity by transforming extraction cells into mini-chromatography columns has been used to demonstrate schemes for the enrichment of high value chemicals from natural product matrices. This can also be accomplished using parallel extraction devices previously designed for multi-sample SFE. Finally, analogies will be drawn with the current state of the art in analytical critical fluid instrumentation and combinatorial approaches being used in the pharmaceutical and materials science fields. Selective reaction chemistry (including analytical derivatization methods) using fluorinated and silylated compounds, suggests that a variety of processes could be accomplished and evaluated in critical fluids using the above methodology. Such an approach would have significant ramifications for propagating "green" technologies.

INTRODUCTION

The development of analytical instrumentation for supercritical fluid extraction started in the late 1980's and has had a complex history. Equipment for this purpose idealistically was to embody a number of features: a wide pressure, temperature, flow rate and sample size range; collection (depressurization) options; cosolvent delivery capability; ability to interface to other analytical instrumentation; and automation. The desire to merger analytical supercritical fluid extraction (ASFE) with its chromatography analog[1] initially resulted in the generation of equipment generically referred to as on-line ASFE[2]. On-line ASFE, although versatile as demonstrated by its coupling with a myriad of analysis techniques[3], has remained more of an academic curiosity, and has seen limited commercial exploitation and instrumentation options.

Off-line ASFE, in which the extraction or associated operations are performed in a suitable module, became the favored mode throughout the 1990's, and gave rise to a competitive effort that once was represented by over ten instrumentation companies. However, some of the instrumentation developed during this period of time is no longer available for a number of complex reasons; including competition between vendors for a limited market, competing sample preparation techniques, and difficulties encountered when using such instrumentation. Despite these factors, ASFE has been successfully applied for the analysis of pesticides[4], determination of fat and related lipid species[5], drugs[6], and complex natural products[7]. This has resulted in the generation of six standard methods utilizing supercritical carbon dioxide (SC-CO_2) as a replacement for organic solvents[8]; additional standard methods are also under development.

In our laboratory, there has existed for over a decade parallel research programs devoted to process supercritical fluid extraction (SFE) and ASFE[9]. Much of the instrumentation used in this program is self-fabricated and its design has been influential in the development of ASFE. However to encourage utilization of ASFE, we have used commercial instrumentation to demonstrate the usefulness of the methods we have developed[10]. Recently, we have employed such instrumentation for non-analytical applications with considerable effectiveness.

There is considerable merit in considering the use of ASFE instrumentation in support of process development. Among these advantages are the savings in time, cost, and labor; over using scaled up apparatus in the development and optimization of critical fluid-based extraction, reaction,

or fractionation processes. ASFE instrumentation because of its scale, does not require copious amounts of reagents, and hence can be used in cases where substrates or catalysts are available in limited quantity, or are exceedingly expensive. Automated ASFE instrumentation, or modules which permit the rapid processing of multiple samples are available, and can reduce experimentation time and effort considerable, when used in a combinatorial fashion[11].

In this presentation, we shall describe how we have utilized ASFE instrumentation as an aid in process development, and not for their originally intended use in analytical chemistry. Several examples will be cited using available commercial instrumentation, where only subtle changes have been required in the equipment to facilitate its use for optimizing reactions or extraction processes in critical fluid media. Where appropriate, home-built analytical equipment will be described that permit the above objectives to be achieved, as well as scale-up of the studied process.

EXPERIMENTAL

In this section, only general aspects of instrumentation used to perform specific experiments will be described. Specific details on the cited examples will be provided in the Results and Discussion section, in the cited references, or by reference to an associated presentation in this symposium.

It should be noted that ASFE instrumentation generally can be classified according to how it functions: modular versus non-modular, sequential versus parallel operation, and manual versus automatic operation. We have used all of these options in non-analytical applications to be described later. Only in two cases were instruments employed that are no longer commercially available (the Dionex SFE 703 system and the Hewlett Packard HP 7680T extractor). The Dionex 703 module permitted parallel processing of up to 8 samples in real time at pressures up to 70MPa and temperatures of 150°C. Cells from 0.5 - 32 mL could be conveniently used with this instrument and cosolvent capability was available. The operational concept of the Model 703 unit was based on a larger scale parallel extraction unit developed at NCAUR and the FDA laboratory in Lenexa, KS[12-13], but restrictor problems limited the Model 703's analytical applicability. The scaled up prototypes of this unit employed a low cost Haskel liquid booster pump and up to 8 extraction cells, ranging in volume from 50 - 140 mL, held simultaneously in a large thermostatted oven.

The defunct HP Model 7680T extractor continues to be used in our laboratory, as well as many others throughout the world. The Model 7680T used in our experiments is conveniently interfaced with a Hewlett Packard gas chromatograph through a "bridge" interface/software package, permitting direct analysis of extraction or reaction products. A carousel of 8-7 mL cells has been used in the described studies over the temperature range of 40 - 80°C and up to pressures of 40 MPa. A variable restrictor nozzle has worked well for continuous, self-compensating flow control, and on-line sorbent traps consisting of stainless steel balls, or a Hypersil ODS packing, have proven facile in trapping analytes prior to transfer to the GC unit. The cosolvent pump accompanying the Model 7680T has been primarily used in the described application to deliver a reactant alcohol, methanol, to the extraction chamber in performing transesterification reactions[14].

Manually operated instrumentation used in performing the described research has consisted primarily of two modules: the Isco Model SFX 2-10 unit (Isco, Inc., Lincoln, NE) and the Spe-ed unit of Applied Separations (Applied Separations, Inc., Allentown, PA). A variety of configurations of the modular equipment comprising the basic SFX 2-10 module have been used in several studies. The base fluid delivery system has been either 2-Model 100 DX pumps (70 MPa capability), or 2-Model 260 D pumps (52 MPa rating), with usually a 100 DX pump utilized for the delivery of reactants into the system. Both PEEK and stainless steel cells have been utilized for non-analytical applications, ranging in size from 0.5 - 10 mL. The versatility and modularity of the Isco manual system has proven to be invaluable in our research, and in one case[15], has involved a complex 2-step synthesis scheme incorporating a total of 4 syringe pumps.

An alternative manually-operated system is the Spe-ed unit, which is based on equipment originating in USDA laboratories[16]. Several of the described experiments have been performed on these units, or units that have been modified to use the component pumping or oven system. The 4 Spe-ed systems in our laboratory have been used in the following modes for:

supercritical and near critical (LCO_2) extraction
solubility experiments in pure and binary fluids
subcritical water extraction, reaction, and solubility measurements
batch and flow reaction studies

Experiments have been conducted with such units up to 70MPa and 150°C; and the pumping systems

slightly modified to deliver binary fluid mixtures[17] and pressurized water, in addition to CO_2. It should be noted that on the Spe-ed units, and all other equipment mentioned in this presentation; only pure CO_2 is used with such units, since helium headspace-CO_2 has been shown to effect solute and reactant solubilities[18]. Various extraction and reaction cell configurations used with the Spe-ed unit will be described in detail in the Results and Discussion section.

The Isco Model SFX 3560, an automated, sequential ASFE unit, designed for high sample throughput in analytical chemistry, has also proven applicable in support of process development. Depending on the syringe pump units servicing the instrument, critical fluid experiments up to 70 MPa and 150°C can be performed. In most of the reported experiments, 2 - 100 D syringe pumps were used to deliver the CO_2 and cosolvent (reactant), respectively. The Model SFX 3560 is microprocessor controlled unit, permitting an array of extraction conditions to be varied from one run to the other. Therefore, with the 24 cell capacity abridged by an equal number of collection vials; and automatic flow control compensation, and temperature control of the collection conditions; it is possibly to rapidly survey a number of combinations of such experimental parameters as pressure, temperature, fluid flow rate, extraction (reaction) time, and variable addition of cosolvent. For most of experiments reported in this presentation, 10 mL cells were used to contain the matrix to be extracted, or an adsorbent or catalyst, when performing the critical fluid-based experiment. As will be shown later, we have used the Model SFX 3560 to optimize the extraction conditions for complex natural product matrices, as a simple supercritical fluid chromatograph by packing the extraction cartridges with a variety of sorbent types, and as a reactor module to test the feasibility and effectiveness of reaction and catalyst, respectively, under supercritical fluid conditions.

RESULTS AND DISCUSSION

Applications of the above-described analytical supercritical fluid instrumentation to numerous studies in our laboratories are listed in Table 1. In addition to the equipment listed, we have also used a fluid recirculation-based apparatus, called a SPA (Sample Preparation Accessory). This unit, which is no longer produced by the Milton Roy Company (Riviera Beach, FL), has been used to determine the effectiveness of a variety of catalysts for accelerating simple esterifications and the reaction enantioselectivity provided by enzymes in the presence of circulating SC-CO_2. Others

have used a similarly-modified apparatus to measure equilibrium solubilities of a variety of solutes in compressed CO_2[19,20]. Likewise, the measurement of solute solubilities can be performed by most of the instrumentation listed in Table 1, but difficulties can arise when using solutes that are solid and semi-solid upon decompression.

Table 1. Examples of analytical instrumentation utilized in non-analytical applications involving critical fluids.

Instrument	**Application**
SFX 2-10	Optimization of Taxol Extraction from Yew Wood
SFX 2-10	Study of Simple Enzyme Esterification Reactions
SFX 2-10	Sterol Ester Fractionation Using Sorbents
SFX 2-10	Feasibility of Aceylated Tocopherols
SFX 2-10	Enzymatic-Initiated Synthesis of Sterol Esters
HP 7680T	SFE and Methylation of Phospholipids and Steryl Esters
HP 7680T	Evaluation of Enzyme Catalytic Activity in SC-CO_2
SFX 3560/Spe-ed	Optimization of SFE of Cedarwood Oil in SC-CO_2 and LCO_2
SFX 3560	Sorbent Selection for Preparative SFC of Phospholipids
SFX 3560	SFE/SFC for Enrichment of Steryl Esters from Corn Bran
SFX 3560	SC-CO_2 Extraction of Pheromone Components from Fir Needles
SFX 3560	Selective Extraction of Components from RBO Deodorizer Distillate
SFX 3560	Optimization of Enzymatic Hydrolysis of Fat Soluble Vitamins
SFX 3560	Sterol and Steryl Ester Enrichment from Corn Bran Oil
SFX 3560	Feasibility of Enzymatic-Initiated Acetylation of Cedrol
Spe-ed	Enzyme Enantioselectivity Studies in SC-CO_2
Spe-ed	Solute Solubility Studies in Subcritical Water
Spe-ed	Batch, Stirred Cell - Ferrulate Ester Synthesis
Spe-ed	Solubility Studies in Binary Fluid Mixtures
Spe-ed/SFX 2-10	Flow Reactor Studies - Hydrogenation/Transesterification
Spe-ed	Subcritical Water Extraction

Space precludes a detailed discussion of all of the examples listed in Table 1, however we shall discuss several selected cases and comment briefly on some of the others. A system such as the Isco SFX 2-10 allows rapid assessment of the conditions required to extract a target solute from a specific matrix. For the case of the anti-cancer compound, taxol, at parts-per-million in yew heartwood as well as elevated levels in the tree's bark, the SFX 2-10 was utilized to survey appropriate SFE conditions. As shown in Table 2, neat carbon dioxide at all pressures and temperatures was of limited effectiveness in removing this compound from bark samples, however addition of a cosolvent methanol at 20 volume percent proved efficacious in maximizing the taxol yield. Using this mixture, over 85% of the taxol, present at lower levels in yew heartwood, could be recovered.

Table 2. SFE of taxol from yew bark and heartwood.

Cosolvent	**Weight % Taxol**
0%	0.0012 %/bark
5% Methanol	0.0032 %/bark
10% Methanol	0.0051 %/bark
20% Methanol	0.0101 %/bark
10% Ethanol	0.0058 %/bark
10% Acetonitrile	0.0057 %/bark
20 % Methanol	0.0006 %/heartwood

The Isco SFX 2-10 system has also been employed to test the feasibility of doing simple esterification and transesterification of oils in SC-CO_2[21]. Using Novozym SP 435 in the SFX 2-10 extraction cells has permitted the successful synthesis of flavor and oleochemical esters. Methyl esters can be readily formed quantitatively using this approach, and this reaction in SC-CO_2[22] has been successfully exploited for analytical purposes as well. These types of reaction have also been accomplished in a batch mode in the presence of SC-CO_2 using a modified laboratory magnetic stirrer placed in the oven of a Spe-ed unit. Similarly, such enzyme-initiated reactions can also be run

on dedicated ASFE apparatus, such as the Isco Fast Fat HT analyzer and the Leco FA-100 fat analyzer (Leco, Inc., St. Joseph, MI).

Other reaction possibilities can be surveyed using an Isco SFX 2-10, or similar instrumentation. Currently acetylation of alpha-tocopherol with acetic acid/anhydride has been attempted using a polymeric amine catalyst (bead form) in a mini (0.5 mL) extraction cell. Acetate (vitamin E) yields of up to 40% have been achieved at relative low pressures by studying the conversion dependence on reaction temperature, CO_2 flow rate, time of reaction, and static hold time. A detailed study has also been performed of the enyzme-initiated synthesis of sterol esters in both the batch and continuous flow mode in SC-CO_2 using Isco SFX 2-10 components. The resultant flow reaction system is shown in Figure 1, where it can be seen that two different syringe pumps are used to solubilize the two reactants in SC-CO_2. These reactants are then mixed together prior to their introduction to the SFX 2-10 extraction cell containing the enzyme catalyst. Different lipases were screened for their effectiveness in SC-CO_2 for the model reaction between palmitic acid and cholesterol. Pressures between 20-31 MPa and temperatures of 40-60°C, over a four-fold change in flow rate, were used to optimize the above reaction. Lower pressures and temperatures were found to yield conversions between 90-100%, provided a 5 minute static hold time was used in conjunction with the flow reaction mode. Chirazyme L-1 proved the best catalyst for the synthesis of number of cholesterol and sitostanol esters of varying acid chain length, again producing yields of over 90%.

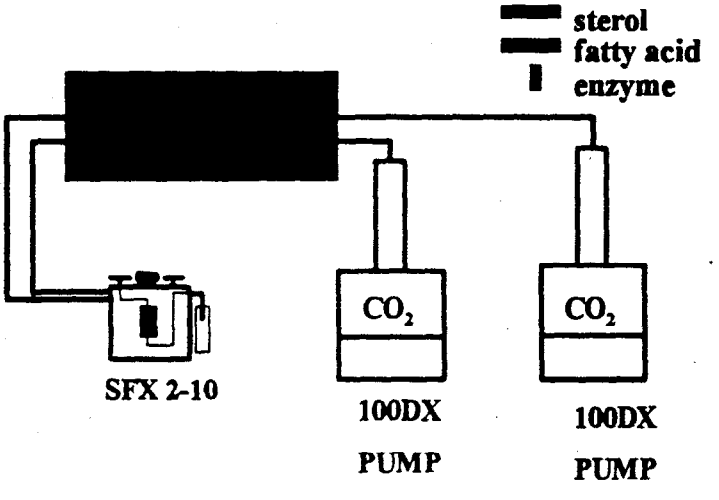

Figure 1. Continuous sterol ester synthesis system using SC-CO_2 as a reaction medium.

Similarly, we have also used the SFX 2-10 system to model the enrichment of sterol esters from vegetable oils[22], using a two-step extraction process coupled with an NH_2-bonded sorbent. Initially, SC-CO_2 at 55 MPa and 80°C is used to remove over 95% of triglycerides, followed by the use of a cosolvent, such as methyl t-butyl ether (10 volume % in CO_2), at 41.4 MPa and 80°C, to elute the concentrated sterol esters from the sorbent. Table 3 shows that significant enrichments of the sterol ester content can be affected by collecting the fractions eluting with the aid of the cosolvent, over those present in the initial vegetable oil.

Table 3. Concentration of sterols in seed oils by supercritical fluid fractionation.

Seed Oil	**Initial %**	**% After Fractionation**
Canola	0.7	29.7
Corn	0.2	21.2
Cottonseed	0.3	30.9
Soybean – SFE	0.2	24.9
Soybean – Hexane	0.1	18.5

In some cases, the use of two instrumental modules in support of process development has proven advantageous. The optimization of the SFE of cedarwood for its high value oil has been reported previously by us[23], primarily utilizing the automated Isco Model 3560 unit. A combinatorial array of parameters, such as pressure, temperature, extraction time, moisture level of wood, and age of the samples were examined to choose the best conditions for extraction on this natural substrate. Use of the Model 3560 proved invaluable, reducing the time and cost of studying these parameters on the resultant extraction. Recently, extraction of cedarwood oil (CWO) has been extended into the near critical range by using a Spe-ed unit for pressurized CO_2 extraction, above and below the critical temperature of CO_2. Table 4 shows the extraction yields from cedarwood for 11 different pressure temperature combinations for passage of 80 liters of expanded CO_2 through 10 grams of wood held in a 50 mL extraction cell contained in the Spe-ed oven. At the lowest pressure tested (6.2 MPa and 25°C), there was essentially no cedarwood oil extracted. This low yield of CWO is undoubtedly a

result of the very low density and solvating power of CO_2 at these conditions. However, at a pressure as low as 10.3 MPa and 25°C, the overall yield of CWO was 3.5%. This indicates that liquid CO_2 can extract CWO effectively at densities as low as ca. 0.84g/mL. At 25°C, and pressures of 19.0 and 41.4 MPa, only slight increases in total yield were recorded. Furthermore, at pressures of 19.0 MPa or 41.4 MPa, the total yield did not vary significantly (ca. 3.5-3.7%) over the temperature range tested (25 - 100°C). This would indicate that CWO can be just as effectively extracted using near critical CO_2 as SC-CO_2. Further details concerning this study can be found in the symposium proceedings[24].

Table 4. Overall yields from cedarwood for various temperature/pressure combinations of CO_2.

Temperature (°C)	Pressure MPa)	Total Yield (wt %)
25	6.2	0.02
25	10.3	3.5
25	19.0	3.6
25	41.4	3.7
40	10.3	3.4
40	19.0	3.7
40	41.4	3.6
70	19.0	3.6
70	41.4	3.5
100	19.0	3.5
100	41.4	3.6

Previously we have reported an enrichment scheme for isolating phospholipids from soybeans using a combination of selective SFE coupled with preparative SFC[25]. Screening of an array of sorbents for the SFC step was accomplished by converting the Model 3560 into a semi-preparative chromatograph by inserting the sorbents into the extraction cells. The microprocessor programming capabilities of the Model 3560 were then brought into play by introducing a series of step gradients of increasing elutropic strength to selectively concentrate individual phospholipids from the initial phospholipid-enriched fraction which was obtained from neat SC-CO_2 deoiled soybean flakes. A

similar scheme has recently been utilized for the enrichment of sterols and sterol esters from corn bran and is reported in this symposium[26]. Again, as shown in Table 5, the Model 3560 can be highly effective in ascertaining optimal conditions for concentrating these high value nutraceutical agents from corn bran. For the initial SFE step, the extraction conditions of 34.5 MPa and 40°C proved optimal for isolating the fatty acid-phytosterol esters (FASE) from the corn bran on mass fraction basis. These conditions were then used for pilot runs in which a larger quantity of corn bran oil was isolated for the SFC stage.

In a similar study, the Model 3560 was put to use in determining whether SFE could be as effective as liquid hexane extraction in isolating pheromone components for flea beetles from needles of the Nordman fir tree. Extractions conducted on a small quantity of fir needles showed that SC-CO_2 at 19 MPa and 50°C was as effective as hexane extraction with respect to the removal of the bio-active components, consisting of sesquiterpene hydrocarbons and sesquiterpene alcohols. Future plans call for using these conditions to extract up to 30 kg of the fir needles.

Not all survey studies done on analytical SFE instrumentation yield positive results. For example, attempts to ascertain selective SFE conditions for the enrichment of sterols and sterol esters from rice bran deodorizer distillate indicated limited enrichment. Likewise, a reaction between acyl donors and one of the principle and valuable components of cedarwood oil, cedrol, was unsuccessful in the presence of SC-CO_2 at several pressure, temperature, and enzyme combinations. Nevertheless, this type of result is valuable, and saves considerable time and effort pursuing processing possibilities that will not work in critical fluid media.

As mentioned previously, Spe-ed units have proven valuable in many studies conducted in our laboratories as generic fluid delivery sources and as a manifold to conduct a variety of operations involving critical fluids. An example of this versatility is shown in Figure 2 where a complex 2-step reaction system is illustrated. In this case, methyl esters of soybean oil are synthesized using the previously reported methods by incorporating 4-syringe pumps along with an Isco SFX 2-10 module. The synthesized esters are then transported in SC-CO_2 into a second reactor stage in which they are exhaustively hydrogenated to give saturated fatty alcohol mixtures. This latter step is accomplished by placing a miniature hydrogenation catalyst bed in the oven of a Spe-ed system, and by using a sequence of tubing and valves, collecting the resultant product after decompression through the

Table 5. Weight % and Mass Recoveries of Components From the SFE of Corn Bran

Compound	13.8 MPa/80°C		13.8 MPa/60°C		13.8 MPa/40°C	
	Weight %	Mass (mg)	Weight %	Mass (mg)	Weight %	Mass (mg)
FASE[a]	19.85	0.10	6.89	0.40	3.92	3.41
TG[b]	53.40	0.27	60.95	3.54	90.11	78.49
FFA[c]	23.68	0.12	28.23	1.64	4.04	3.52
FS[d]	2.40	0.01	3.19	0.19	1.10	0.96
FPE[e]	0.67	0.01	0.74	0.04	0.83	0.72
	34.5 MPa/80°C		34.5 MPa/60°C		34.5 MPa/40°C	
	Weight %	Mass (mg)	Weight %	Mass (mg)	Weight %	Mass (mg)
FASE	3.10	2.79	3.60	3.75	3.15	3.46
TG	87.40	78.40	87.67	91.44	90.91	100.09
FFA	3.83	3.44	4.88	5.09	3.57	3.93
FS	4.62	4.14	2.73	2.85	1.15	1.26
FPE	1.04	0.94	1.12	1.17	1.23	1.35
	69 MPa/80°C		69 MPa/60°C		69 MPa/40°C	
	Weight %	Mass (mg)	Weight %	Mass (mg)	Weight %	Mass (mg)
FASE	4.10	4.32	3.48	3.24	3.67	2.99
TG	88.96	93.86	88.16	81.99	87.26	71.11
FFA	4.54	4.80	5.40	5.02	3.55	2.89
FS	1.13	1.20	2.05	1.91	4.44	3.62
FPE	1.26	1.33	0.92	0.85	1.09	0.89

a) FASE = fatty acid-phytosterol esters, b) TG = triglycerides, c) FFA = free fatty acids, d) FS = free sterols, e) FPE = ferulate-phytosterol esters

pictured micrometering valves. Binary fluid mixtures of CO_2 and H_2 are generated in the desired molar proportions by using a home-built system consisting of two mass flow controllers coupled with a 1 liter stirred autoclave, the contents of which are fed to a gas booster pump for compression to the desired pressure. Details of this system are available in the literature[27], including the design of the apparatus for generating binary fluid mixtures[17].

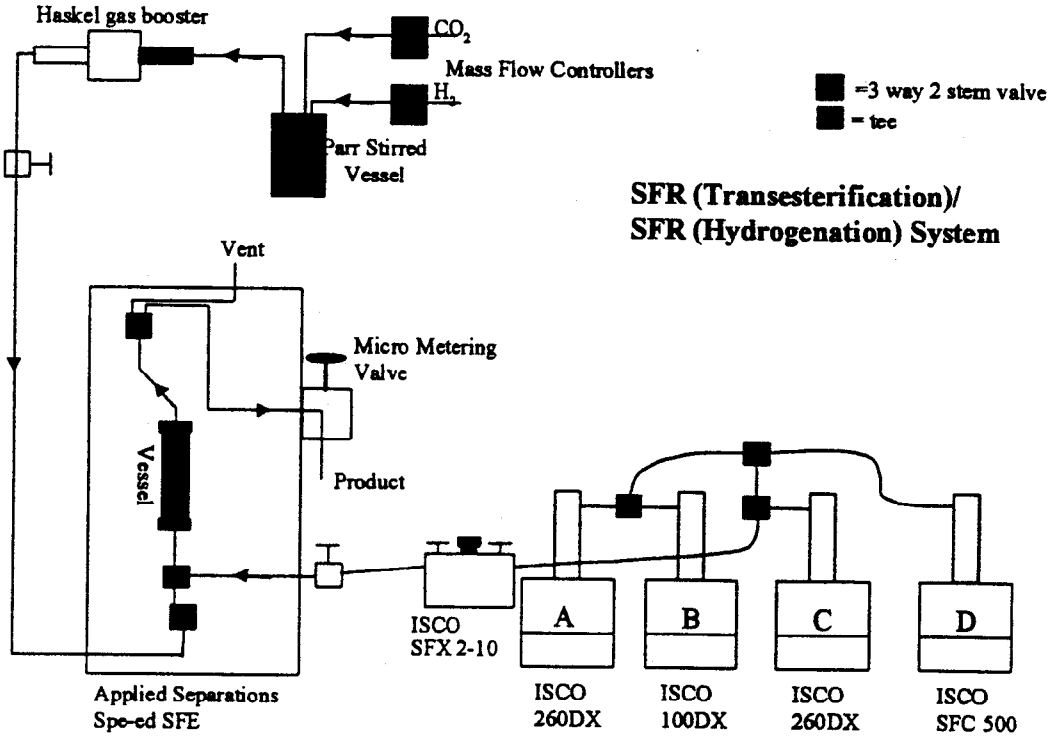

Figure 2. Two-step reaction system incorporating components of an Isco SFX 2-10 unit with a Spe-ed unit/NCAUR fluid mixing module.

As reported in these symposium proceedings[28], batch reactions in stirred cells can be conveniently studied in the presence of SC-CO_2 by inserting a novel reaction cell designed by Jeff Teel of our research group. The reaction cell consisted of a modified pressure gauge snubber (Part No. 30-31HF4, Chemiquip Products Co., Inc., New York, NY). The modified gauge snubber proved to be an excellent reaction cell due to its flat bottom and sufficient internal volume which allowed

for the inclusion of a small magnetic stirring bar in the cavity. The reaction cell was placed on top of Lab-Line Magnestir (Part No. 1250), which was then placed in the Spe-ed oven to facilitate mixing in the dense CO_2 phase. The enantioselectivity of the reaction between 3-methyl-2-butanol and vinyl octanoate was then carried out at pressures from 14-22 MPa over a temperature range of 45-90°C and the enantioselectivity parameter, E, determined using Novozym SP 435 as a catalyst.

Figure 3 shows the results of the experimental measurement of E versus temperature for the reaction. As indicated in Figure 4, enantiomeric enrichment of the ester product is best achieved at the lower temperatures over the range studied, and ranges from approximately 225 at 45°C to a value of 100 at approximately 85°C. These E values are somewhat lower than those obtained for the same reaction conducted in organic solvents. The same experimental apparatus has recently been utilized for studying the reaction between ethyl ferrulate and 1-octanol to produce an ester with sun screening properties. Using Novozym SP 435 at 20.7 MPa, resulted in conversion rates that increased with temperature over the range of 40-80°C; the highest conversion (approx. 55%) being achieved at 80°C after 48 hours.

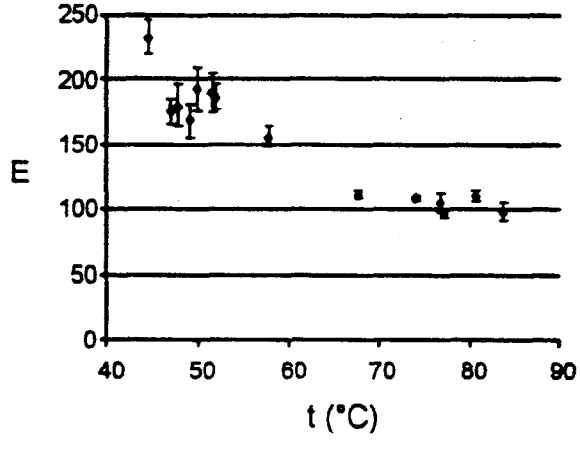

Figure 3. Variation in E with temperature for the transesterification of 3-methyl-2-butanol with vinyl octanoate in SC-CO_2 as obtained with a modified Spe-ed unit.

A similar apparatus and approach employing a Spe-ed unit has recently been used to measure solute solubilities in subcritical water. The apparatus is shown in Figure 4 below and utilizes the same pressure gauge snubber cited above. An Isco pump is used to deliver pressurized water (5 MPa)

to the solubility cell, which is held in the Spe-ed unit's oven. A special stirrer (Variomag Telesystem Micro HP 40107, Daytona Beach, FL) is used to facilitate stirring in the cell. For sampling, a 6-port valve (Valco #6C6WEY, Valco Instruments Co, Inc., Houston, TX) is turned to the "load" position and the shut-off valve is opened. The 6-port valve is then switched to the "inject" position and the shut-off valve is closed. The samples from the cell were diverted to an adjacent HPLC system using a reverse phase column and mobile phase consisting of 70% acetonitrile and 30% water with detection conducted at a UV wavelength of 225 nm. The temperature of the solubility cell was measured with a Type J surface mounted thermocouple. Depending on the solute, equilibrium solubilities were established in times varying from 30 to 120 minutes. Solubilities measured by Dr. Meredith Curren in our laboratory for naphthalene were as follows (Table 6).

Table 6. Solubility measurements of naphthalene in water conducted on a modified Spe-ed system.

Temperature(°C)	Pressure(MPa)	Solubility (ppm)	Ref 29 value
25.7	4	39 +/- 2	34 +/- 1
25.0	0.1	36 +/- 1	36 +/- 1
35.4	4	69 +/- 2	49 +/- 3
50.7	7	128 +/- 6	101 +/- 5
65.8	3	263 +/- 9	216 +/- 8

The determined solubilities show a strong dependence on temperature and are in reasonably good agreement with the values quoted by Miller and Hawthorne[29].

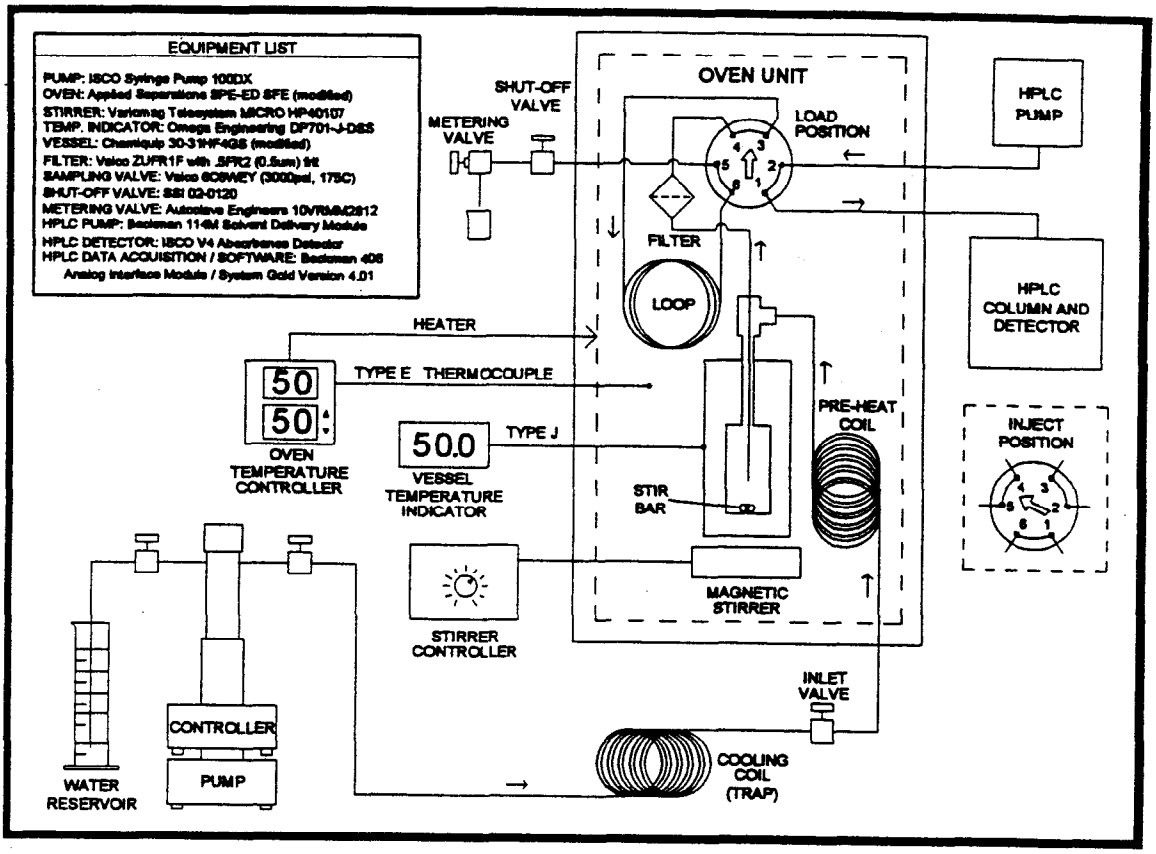

Figure 4. Spe-ed-based apparatus for measuring solute solubilities in subcritical water.

It should be noted that the Spe-ed unit shown in Figure 4 can be used as an extraction unit utilizing the Spe-ed unit's liquid booster pump to deliver water to an extraction cell. This conversion was facilitated by Dr. Russell Holliday and is one of two flow extraction/reaction units for use with subcritical water at NCAUR. The converted Spe-ed unit at NCAUR is rated for operation at 48.3 MPa and 300°C and is similar in configuration to a Spe-ed unit for use with SC-CO_2 except the shut-off valves and micrometering valves have been mounted, distended from the outside oven surface. The subcritical water extraction of pesticides from food matrices has been accomplished using this extractor design.

CONCLUSIONS

The batch, sequential, and parallel extraction/reaction equipment described in this manuscript provide a powerful arsenal for investigating critical fluid phenomena. The ability to study extractions, fractionations and reactions in critical fluid media with the aid of the described analytical instrumentation considerably reduces the effort and expense associated with investigations prior to considering scale up of a process. Particularly powerful are the sequential or parallel cell arrangements that have been described, since they facilitate a combinatorial approach to optimizing processing with critical fluids[30]. ASFE equipment can play a key role in a critical fluid technology program and augment process engineering studies, which ultimately will lead to the commercial acceptance of this versatile technology.

DISCLAIMER

Names are necessary to report factually on available data; however the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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